

TITLE OF THE INVENTION

5 LIGHT-INDUCED ELECTRON CAPTURE AT A SURFACE

CROSS REFERENCE TO RELATED APPLICATIONS

U.S. provisional application no. 60/139,170, filed June
15, 1999 and U.S. provisional application no. 60/138,466,
10 filed June 10, 1999

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

Support came from Health Effects Institute, Cambridge,
15 MA, which is itself funded at least in part by EPA.

BACKGROUND OF THE INVENTION

This invention relates to attaching an electron to a
chemical substance on or covalently attached to a solid or
20 particulate surface, especially to ionize and desorb the
substance for detection by mass spectrometry.

Mass spectrometry (MS) is a powerful technique for both
quantitative and qualitative chemical analysis. Typically
(but not always) there are three sequential stages for the
25 analysis: sample volatilization/ionization, ion separation,
and ion detection. One form of volatilization is called
laser desorption (LD); one form of ionization is called
electron capture (EC); and time-of-flight (TOF) is a form of
ion separation.

The combination of LD, EC and TOF has been known before, but this invention relates to the recent discovery that certain conditions allow the LD and EC options for MS stages to be combined in a practical way, especially with the TOF option, for a diversity of analytes to give high sensitivity and resolution. The novel procedure has been named "laser induced electron capture mass spectrometry" (LI-EC-MS).

10 Abbreviations

LD-TOF-MS: laser desorption time-of-flight mass spectrometry

IP: Ionization Potential

LI-EC-MS: laser induced electron capture mass spectrometry

15 LI-EC(Ag)-TOF-MS: LI-EC-MS on a silver surface in a time-of-flight mass spectrometer.

LI-EC(matrix)-TOF-MS: LI-EC-MS with a matrix in a time-of-flight mass spectrometer.

20 LI-EC(Ag)-FTMS: LI-EC-MS on a silver surface in a Fourier transform mass spectrometer.

LI-EC(In)-TOF-MS: LI-EC-MS on an indium surface in a time-of-flight mass spectrometer.

In the photoelectric effect, light is used to eject an electron from a metal surface into a vacuum. The event is characterized in part by the work function of the metal, which is the minimum energy required to eject an electron. Such an ejected electron is called a "photoelectron". Thus the wavelength of the light must correspond to an energy which is greater than or equal to the work function to

25
30

produce a photoelectron. For example, this wavelength is 263 nm for a silver surface (J.D. Cutnell and K.W. Johnson, Physics, Wiley and Sons, New York, 1998, p. 893). Einstein was awarded the Nobel prize in physics in 1921 largely because he explained the photoelectric effect. The work function of a metal can be changed by placing a charge on the metal with a voltage.

Lasers are applied to a metal surface for several purposes, e.g. to produce photoelectrons, to warm the surface, to cut into or drill through the metal, and to desorb a substance off the surface. The latter technique is especially useful in mass spectrometry as a way to volatilize an analyte off the surface for detection, and is referred to as "laser desorption mass spectrometry" (LD-MS). The analyte on the surface may have a charge in the first place, or acquire a charge due to the desorption event. Often a secondary substance termed "matrix" is present on the surface as well, to facilitate the desorption of the analyte. The matrix minimizes photo or pyrolytic destruction of the analyte, and usually modifies the charge of the analyte during desorption. Often the latter event involves the uptake or loss of a proton by the analyte, or the uptake of an alkali metal ion (e.g. Na^+) or halide ion (e.g. Cl^-), due to interaction of analyte with the matrix and other substances in the matrix.

Another way in which an analyte on a metal surface has been ionized with a laser for detection by mass spectrometry involves the transfer of an electron to a neutral analyte. This initially converts a neutral analyte into an anion radical, and the event may cause the analyte to fragment,

producing anionic and neutral products. In MS techniques developed in the past, it has been reported that the electron for the event can come from one of two sources: (1) another analyte molecule or a contaminant on the surface, and (2) the metal surface.

For example, Reilly and coworkers (Colby, S.M., Harris, W.A., Hanser, K.H., and Reilly, J.P., Photoelectron Capture Negative Ion Mass Spectrometry of Organohalogens, 43rd ASMS Conference on Mass Spectrometry and Allied Topics, 1995, p. 1021) detected anionic fragmentation products of octafluoronaphthalene and o-dichlorobenzene deposited as vapors (gaseous state) on a stainless steel surface by LD-MS in which 6.4 eV photons were used to generate photoelectrons. Routine LD-MS equipment is not outfitted to handle vapor samples. Kelly and coworkers (Bezabeh, D.Z., Allen, T.M., McCauley, E.M., Kelly, P.B., Jones, A.D., Negative Ion Laser Desorption Ionization Time-of-Flight Mass Spectrometry of Nitrated Polycyclic Aromatic Hydrocarbons, J. Am. Soc. Mass Spectrom., 8, (1997) 630-636) detected anionic products of nitrated polycyclic aromatic hydrocarbons deposited on an aluminum or silica surface by LD-MS using 266 or 213 nm laser photons, and considered that the ionizing electrons came from the deposited sample, consisting of analytes plus contaminants, in the gas phase.

Ehring et al. (Ehring, H., Karas, M., and Hillenkamp, F., Role of Photoionization and Photochemistry in Ionization Processes of Organic Molecules and Relevance for Matrix-assisted Laser Desorption Ionization Mass Spectrometry, J. Mass Spectrom., 27 (1992) 472-480) reported the detection of 7,7,8,8-tetracyanoquinodimethane (a solid) as a molecular

anion radical species by LD-MS, although the exact conditions were not defined. In the overall study, 266 or 337 nm photons were used, along with a silver or steel surface. The latter were cited as "inert surfaces" and were not distinguished in their role. This implied that the electron for electron capture came from one analyte molecule (acting as a matrix) donating to another. Indeed, the study focused on photoionization and photochemistry mechanisms of matrices, with the photons considered to act directly on the matrix, and with the metal surface being inert.

In conventional electron capture mass spectrometry, a gas phase analyte is ionized by combining with a gas phase electron. Ordinarily primary, high energy electrons are emitted from a hot wire, and collide with a dilute gas (e.g. methane) to create secondary, low energy electrons that are the ones captured by the analyte. A common sequence of events is to derivatize an organic compound with a polyfluoro organic reagent (e.g., pentafluorobenzyl bromide, o-(pentafluoro-benzyl)hydroxylamine, 3,5-bis-(trifluoromethyl)benzyl bromide, heptafluorobutyric anhydride, trifluoroacetic anhydride, or pentafluorophenyl sulfonyl chloride) and perhaps other reagents, and subject the product to gas chromatography electron capture mass spectrometry. Polyfluoro organic reagents also have been introduced that can be attached to macromolecules such as protein or DNA, and then the labeled macromolecule is detected by chemically or physicochemically releasing a polyfluoro organic molecule in a volatile form for detection by gas phase electron capture (Bian et al, Rapid Commun. Mass Spectrom. 11 (1997) 1781). Hurst and coworkers (Britt,

P.F., Hurst, G.B., and Buchanan, M.V., Synthesis of Electrophore-labeled Desorption/Ionization Mass Spectrometry, J. Mass Spectrom., 31 (1996) 661-668) attempted, without success, to enhance the detection of an oligonucleotide by derivatizing it with a polyfluoro organic reagent, and subjecting the product to LD-MS in the presence of a conventional matrix functioning as an electron donor, where no effort was made or suggested to employ a matrix with a low ionization potential. Instead it was assumed that the experiment failed because electrons were produced with too much energy to enable electron capture. This interpretation implies that a matrix with a low ionization potential would be counterproductive, contrary to part of our discovery.

Polanyi and coworkers (Dixon-Warren, St. J., Jensen, E.T. and Polanyi, J.C. Direct Evidence for Charge Transfer Photodissociation at a Metal Surface: CCL/Ag(111), Physical Review Letters, 67 [1991] 2395-2398; Ibid., Photochemistry of Absorbed Molecules. XI, Charge-transfer Photodissociation and Photoreaction in chloromethands on Ag(111), J. Chem. Physics 98[1993] 5938-5953) reported that Cl^- ions were emitted when a gaseous sample of CCl_4 , CHCl_3 , CH_2Cl_2 , CH_3Cl or CCl_3Br was adsorbed on a cold (near 100°K) polished silver surface, and photons were applied, even when the photon energy was slightly below the work function of the silver. The Cl^- ions were detected in a mass spectrometer. To the best of our knowledge, no broadening of the method to other analytes, aside from comparable ones such as hydrogen halides, has ever been reported. This may have been discouraged by (1) the failure to observe any Br^- ions from

CCl₃Br, (2) the use of exotic equipment and conditions, including a very low power density of photons (about 1 $\mu\text{J}/\text{cm}^2$), (3) the very low resolution (broad peaks) observed, (4) the very low signal/noise when photons below the work function of silver were used, and (5) the failure to observe any signal with photons having an energy below 4.1 eV, indicating that the technique cannot be practiced on a silver surface with low-cost, common lasers such as nitrogen (photon energy 3.68 eV) and neodinium: YAG (photon energy 3.5 eV) lasers. Further, the sharp reduction in signal when the thickness of deposited sample was greater than 1-3 molecules thick may have discouraged the testing of samples deposited in a non-gaseous state, such as liquid drops, droplets or spray. Mass spectrometry equipment with cold sources is not in general use.

BRIEF SUMMARY OF THE INVENTION

In one form of our invention, a chemical substance in a non-gaseous state is deposited onto a metal (the term metal referring to either a single metal or a metal alloy) surface and captures an electron from the surface due to activation of a surface electron by light, especially by using laser light, where the energy of the light is below the work function of the surface, and the power density preferably is high. For example, an analyte on a silver surface, especially an analyte which contains a polyfluoro organic group, can be detected by laser desorption mass spectrometry using a nitrogen laser (337 nm), due to the formation and desorption of an anionic product by this novel

technique, where the anionic product may be a fragment of the starting analyte and where the laser energy is below the work function of silver. The work function of silver is 4.26 eV. The analyte may also be a salt, where, in some cases, a neutral component of the anion part receives an electron, leading to the formation and desorption of a different anion, or, in other cases, the cation part is neutralized by the electron, leaving an anion part that is no longer ion-paired with the cation. It is preferred to use a polyfluoro organic neutral component which undergoes dissociative electron capture in the former case, and an organic cation in the latter.

In a second form of our invention, the analyte, which is a first chemical substance on a surface, captures an electron from a second chemical substance, which also is present on the surface, where this electron is activated directly or indirectly by subjecting the surface or second chemical substance to light. If the second chemical substance is activated directly by the light, it should have a low ionization potential. For example, the ionization potential of the second chemical substance should be below 8 electron volts, and preferably below 7.5 electron volts. Examples of substances with low ionization potentials are benzo[ghi]perylene, perylene, tetracene, dimethylaniline, diphenylamine, ferrocene, rubrene, terthiophene and graphite. Preferably the solid or particulate surface supporting the first and second substances is highly absorptive for the photons, such as a non-polished or finely roughened metal or metal particle surface. If the surface is the source of the electron, the surface should be a

metallic (metal or metal alloy) surface. The second chemical substance may be a solid or liquid, and may be combined with additional substances on the surface.

In both forms of our invention, the substance which captures an electron can be an organic substance which is bonded covalently to the surface via a bond that breaks when the substance captures an electron. For example, organic substances can be bonded covalently via sulfur atoms to silver or gold surfaces. One would select a substance containing a moiety which readily undergoes dissociative electron capture for this purpose.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic of the LI-EC-MS technique, with (A) showing formation of the anion version of the analyte coming from the surface, (B) showing the version where the electrons are initially released by the second substance and form the anionic form of the analyte, and (C) shows the version where electrons are released from the surface and form the anion of the second substance, which subsequently releases electrons which can form the anion of the analyte. In both (B) and (C), it should be understood that the first and second substances may be present in different ways on the surface, e.g., mixed together, or one on top of the other.

Figure 2 shows the detection of pentafluorobenzylated estradiol by LI-EC(Ag)-TOF-MS.

Figure 3 shows the detection of 2,3-bis(pentafluorobenzyl) pyrenedicarboxylate by LI-EC(Ag)-TOF-MS.

Figure 4 shows the detection of 4-[4-methoxy-2,3,5,6-tetrafluorobenzyl)oxy]acetophenone by LI-EC(matrix)-TOF-MS, on a stainless steel (A) and gold (B) surface.

Figure 5 shows the detection of two electrophores by LI-EC(Ag)-TOF-MS: 100 pmol on the target, 10 laser shots.

Figure 6 shows:

(A) the detection of a mixture of six electrophores

(structures shown at top) by LI-EC(Ag)-TOF-MS and

(B) LI-EC(Ag)-TOF-MS of a blank silver surface under the same conditions as in (A).

Figure 7 shows:

(A) Detection by MALDI-MS of an oligonucleotide before (a) and after (b) labeling with a reactive form of electrophore II-475a.

(B) Detection by LI-EC(Ag)-TOF-MS of the latter, electrophore- labeled oligonucleotide.

Figure 8 shows detection by LI-EC(Ag)-FTMS of penta-fluorobenzylated estradiol (structure shown in Figure 2).

Figure 9 shows detection of compound 591 by LI-EC(In)-TOF-MS.

DETAILED DESCRIPTION OF THE INVENTION

This invention is a useful improvement in the area of mass spectrometry. First, it provides a way to accomplish electron capture mass spectrometry on a routine laser desorption mass spectrometer. Such instruments ordinarily are fitted with a nitrogen laser, which works nicely with our invention, at least when a silver or indium surface is employed, or when a polyaromatic hydrocarbon with a low

ionization potential is used as an electron-donating matrix in combination with a photon absorbing/scattering surface such as unpolished gold. The photon-absorbing/scattering properties of a surface can be increased by roughening it
5 finely or by using an unpolished surface. Some of these instruments enable a voltage to be applied to the surface, which will modify the work function and may provide a way to tune the surface electron capture.

Second, our invention enables electron capture mass
10 spectrometry to be accomplished without a hot filament to furnish electrons, without a gas such as methane, and without a special electron capture cell with associated electronics, heating and valving. Such cell also requires periodic cleaning. Third, our invention expands the variety
15 of substances that can be detected by electron capture mass spectrometry, since it is no longer necessary to volatilize a substance prior to electron capture. This means that larger and more polar substances can be detected by electron capture mass spectrometry. Fourth, our invention narrows
20 the energy range of the desorbed anions, to increase resolution, relative to some conventional LD-MS techniques with or without a matrix. Fifth, our invention can reduce sample preparation prior to detection by electron capture mass spectrometry, since less derivatization can be used.
25 Sixth, our invention provides a new way to release a molecule from a surface to which it is covalently bonded.

This invention differs significantly from prior electron capture mass spectrometry, where gas-phase electrons are or seem to be employed, or surface-phase (sub-
30 work function) electrons seem to be employed, but only on

gas-phase deposited, neutral compounds on a very cold surface. In no case has the remarkable result been reported before that the peak width for the detected ion in a sub-work function electron capture experiment with laser desorption mass spectrometry in the continuous extraction mode essentially matches the pulse width of the laser. In particular, prior sub-work function electron capture mass spectrometry has provided very broad peak widths, apparently orders of magnitude greater than the laser pulse width, providing such very low resolution as to dissuade any further study of the technique for analytical purposes. Also, the concept of photo-bombarding a substance that has a low ionization potential in order to provide effective electrons for electron capture has not appeared before. In this invention, electrons are provided to the analyte deposited on a surface either (1) from the surface itself, (2) from a second substance or intermediate which is also on the surface, said intermediate getting the energy to give off the electrons directly or indirectly from the photons beamed by the laser, or (3) from the intermediate, which in turn received electrons provided from the surface, when the surface was bombarded by photons.

Our invention takes sub-work function electron capture mass spectrometry from the world of adsorbed methyl halide molecules gas-phase deposited on an extremely cold surface in a special instrument, yielding very low resolution anion signals, into a vastly different regime of achieving high resolution and high sensitivity detection, even of polyfluoro-labeled DNA molecules adsorbed on a room temperature surface in an ordinary mass spectrometer. It is

important that our technique allows non-gaseous substances to be deposited, since many analytes are nonvolatile, and it is difficult to locally deposit the ones that are volatile as a small spot onto a convenient surface.

5 When the surface comprises a metal (i.e., single metal or metal alloy), photons with energy below the work function are used in this invention to activate the surface electrons for electron capture. The work function is known or estimated for numerous materials and can be determined by
10 examining one of several common references (e.g., Handbook of Chemistry and Physics, 72nd edition, 1991-1992, D.R. Lide, ed., CRC Press, page 12-97).

 Generally, in one form of the novel technique, the metal surface on which the analyte is deposited can be
15 chosen from a wide variety of materials, including, but not limited to, silver and indium. (The work function of indium is 4.12 eV.) In the case of the electron donor comprising a second substance with a low ionization potential, a photon-absorbing surface, such as non-polished gold or stainless
20 steel, is preferred. Either kind of surface can be particles (i.e., a particulate surface).

 Our invention works with various types of lasers which have a photon energy less than the work function of the surface. For example, a nitrogen laser (337 nm for the
25 laser, which is equal to 3.68 eV) will work well on a silver surface (work function is 4.26 eV). In addition, on silver, a neodymium YAG laser (355 nm for laser, which is equal to 3.5 eV) will work well. The power density of the photon output of the laser should be equal to or greater than 10⁵
30 watts per square centimeter. Thus we have achieved LI-EC-MS

with photon energies which are more than 0.4 eV below the work function of the surface, and we have achieved LI-EC-MS with photon energies below 4.0 eV. Low photon energies can be advantageous in that they can minimize analyte multi-fragmentation to enhance detection sensitivity.

EC and TOF can be brought together efficiently in this invention in two, related ways, both of which involve the activation of electrons (for electron capture by the analyte) with a laser pulse that in turn initiates the TOF measurement. In one way, the electrons in a metal-containing surface supporting the analyte are activated by a laser pulse. In the second way, the electrons of a matrix surrounding or supporting the analyte are activated, wherein the matrix has a low ionization potential.

When a metal surface directly furnishes an electron to the analyte in our technique, photons are used with too little energy to fully eject electrons off the surface. Related to this, the ions formed can leave the surface during an interval of time which matches the pulse time of the laser (e.g. 4 nsec), or nearly so, an ideal desorption mechanism in order to achieve high resolution.

It is preferred in our technique that the analyte of interest can undergo dissociative electron capture due to its containing an appropriate polyfluoro-containing group, such as a pentafluorobenzyl group or a bis-3,5(trifluoromethyl)benzyl group, each of which is an example of a polyfluorophenyl group, substituted at an appropriate site, such as an acidic OH or NH site, on the analyte. This can even allow our invention to be used to detect many polyfluoro-labeled biomolecules, such as

polyfluoro-labeled proteins, peptides, amino acids, nucleic acids (including DNA and RNA), catecholamines, prostaglandins, sugars and steroids. Polyfluoro-labeled drugs and drug candidates from combinatorial synthesis also
5 can be detected. These and other preferred analytes generally contain one or more H-substituted nitrogen or oxygen atoms, thus making them easy to label with an appropriate polyfluoro organic group.

This method is particularly useful for quantitative
10 analysis of an analyte having a mass of greater than 300 atomic mass units. As can be seen from the figures provided, the analysis of the analytes yielded one or more very sharp peaks, thus helping to minimize interferences in the detection of the analytes.

Our invention potentially is useful in numerous other
15 procedures which utilize a chemical entity acquiring an extra electron, including those that involve the use of photolithographic techniques including masks. For example, our invention can be used for printing or writing, to store
20 or process data, to transmit or store or transduce signals such as photon or electrical or thermal or electrochemical signals, to create images, to conduct chemical reactions for synthetic or mechanistic purposes, to build or operate electrical circuits or components, to create reactivity
25 sites on surfaces for immobilization of chemical, physical or biological substances, to achieve welding or adhesion, to prevent nonspecific binding, to attach a surface film to initiate polymerization, to create a polarization, and to detect photons.

One variation of the invention is when energetic particles with energies below the work function of the surface are substituted for photons. Furthermore, the invention can be enhanced by including electrically
5 conductive substances on the surface, including those that are photon-transparent.

The following examples are intended to illustrate, not limit, the invention.

10 Example 1. Mass Spectrometry Conditions

The LI-EC-TOF experiments were carried out on a Bruker Daltonics Proflex laser desorption TOF-MS instrument in the linear negative mode, using a nitrogen laser (3.68 eV) that gave a power density at the sample in the range of 10^6 - 10^7
15 watts/cm² (attenuation setting = 50; 4 nsec pulse width). Operation parameters were as follows: accelerating voltage 20 kV; grid voltage 18.8 kV with delay time setting "short" (ca. 100 ns) when matrix was used, or 15.6 kV in the absence of matrix with no delay time. The CCA matrix stock for
20 MALDI-MS was a saturated solution of α -cyano-4-hydroxycinnamic acid in 1:1 acetonitrile:water with 0.5% trifluoroacetic acid overall. Benzo[ghi]perylene (BP) or benzo[b]fluoranthene (BF) was dissolved in toluene to give a 0.01 M stock matrix solution. Analytes in ethyl acetate or
25 acetonitrile (or the PAH mixture as supplied) were mixed with matrix (1:1 ratio CCA matrix for MALDI-MS; BP or BF for EC(matrix)-MS) before loading onto the target and evaporation. Silver foil (0.125 mm, Aldrich) was cleaned by sonication in 10 mL 5% ammonia for 1 min, soak in distilled
30 water (2x briefly), and sonication for 1 min in 10 mL

methanol followed by 10 mL ethyl acetate. This removes $[AgCl]_nCl^-$ background ions seen by LI-EC(Ag)-TOF-MS without such cleaning. The foil was attached onto the regular stainless steel target of the TOF-MS with Scotch double stick tape (<0.1 mm thickness, No. 136, 3M Company). It was always easy to find a "sweet spot" (<5 random locations tested routinely), and the signal intensity among the spots sampled usually varied <10-fold. One μ L samples in acetonitrile or ethyl acetate (without matrix) were loaded onto the silver surface. All samples spread to a diameter of about 2 mm on the metal target.

Example 2. Polyfluoro labeling of α -estradiol, giving 3-O-(Pentafluorobenzyl)- α -estradiol, and detection of the latter by LI-EC(Ag)-TOF-MS.

3-O-(Pentafluorobenzyl)- α -estradiol was prepared by vortexing a mixture of α -estradiol (0.1 mmol), K_2CO_3 (0.2 mmol), and tetrabutylammonium bromide (1 mg) in 1 mL of dimethylformamide for 1 hour, then adding pentafluorobenzyl bromide (30 μ L) and 3 drops of water. After stirring for 8 hours at 60°C, product (85% yield) was obtained by flash chromatography on silica with 1:3 ethyl acetate:hexane. Figure 2 shows the detection of pentafluorobenzylated estradiol by LI-EC(Ag)-TOF-MS. Sample deposition: 10 pmol in 1 μ L of ethyl acetate giving a 2 μ m spot. Laser spot size: 100 μ m (0.25% of sample). Number of laser shots: 50. S/N = 50. See Example 1 for MS conditions for LI-EC(Ag)-TOF-MS.

Example 3. Analysis of 2,3 - Bis(pentafluorobenzyl) pyrenedicarboxylate

2,3-Bis(pentafluorobenzyl) pyrene-dicarboxylate was prepared as described (Allam, K.; Abdel-Baky, S. and Giese, R.W., Anal. Chem. 1993, 65, p. 1723) and 10 pmol was placed on the target. Using the conditions for LI-EC(Ag)-TOF-MS described in Example 1, sixty laser shots gave the data shown in Figure 3. The fragmentation pattern is independent of the number of laser shots. We postulate that the compound exhibits significant fragmentation since the pyrene moiety is absorptive at 337 nm.

Example 4. 4[(4'-Methoxy-2,3,5,6-tetrafluorobenzyl)oxy]acetophenone

4[(4'-Methoxy-2,3,5,6-tetrafluorobenzyl)oxy]acetophenone was synthesized as described (Xu, L.; Bian, N.; Wang, Z; Abdel-Baky, S; Pillai, S; Magiera, D; Murugaiah, V.; Giese, R.W.; Wang, P.; O'Keeffe, T.; Abushamaa, H.; Kumey, L.; Church, G.; Carson, S.; Smith, D.; Park, M.; Wronka, J.; Laudien, F.; Anal. Chem., 1997, 69, 3595-3602). One nmol of this compound in 1 µL ethyl acetate containing benzo[ghi]perylene matrix was deposited onto (A) a polished stainless steel surface and (B) a gold surface as a 2 mm spot size, and detected using the conditions for LI-EC(matrix)-TOF-MS described in Example 1, with 20 laser shots, each 100 µm in diameter, giving the data shown in Figure 4.

Example 5. Analysis of synthetic precursors for electrophore release tags

Ethyl-Isonipecotate was reacted at 70-80°C with isopropylidene-D-erythronolactone, and the product, after
5 purification by Si flash chromatography, was reacted with decafluorobiphenyl in acetonitrile with CsOH and molecular sieves at room temperature to give compound I. In turn, I was reacted with 4'-hydroxyphenyl-2-butanone in the presence of K₂CO₃ and acetonitrile to give I-475a. Using the
10 conditions for LI-EC(Ag)-TOF-MS described in Example 1, 100 pmol of each of these compounds was detected, using 10 laser shots, as shown in Figure 5. These compounds are useful as synthetic precursors for electrophore release tags.

15 Example 6. Analysis of 6 compounds

(A) Using reactions similar to those described in Example 5, the six compounds shown in Figure 6 were synthesized. These analyte compounds are useful as synthetic precursors for electrophoric release tags. A
20 mixture of the six electrophores was prepared as a solution in ethyl acetate and 1 µL (120 fmol of each) was analyzed using the conditions for LI-EC(Ag)-TOF-MS described in Example 1, using 10 laser shots from a 100 µm-diameter laser beam applied to a 2 mm-diameter spot of the sample, giving
25 the data shown in Figure 6A. Note the significant signal to noise ratio, which indicates an extrapolated detection limit in the amol range, since only a small fraction of the sample spot is in the laser beam. (B) LI-EC(Ag)-TOF-MS of a blank silver surface under the same conditions as (A).

Example 7. Analysis of II-475a

Using reactions similar to those described in Example 5, compound II-475a was synthesized as an N-hydroxysuccinimide (NHS) ester and reacted with a 5'-aminoalkyl DNA 20-mer oligonucleotide having the sequence ACCGGCCATGCTCGCGACCA. Detection by matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), using the conditions for this technique that are described in Example 1, of the oligomer before (a) and after (b) reaction with II-475a NHS ester is shown in Fig. 7A. Detection of 500 fmol of the II-475a-labeled oligomer using the conditions described in Example 1 for LI-EC(Ag)-TOF-MS, with 6 laser shots, is shown in Fig. 7B.

Example 8. Analysis of surface-bound compound II-475a

The NHS ester of compound II-475a that is described in Example 7 can be reacted with ethylene diamine, and the resulting product in turn can be reacted with thioctic acid NHS ester. The resulting compound can spontaneously bind covalently to silver, and an anionic fragment of this compound can be detected by conducting LI-EC(Ag)-TOF-MS on the deposited sample using the conditions for the techniques that are described in Example 1.

Example 9. Analysis using a neodymium-YAG laser

Detection of pentafluorobenzylated estradiol by LI-EC-FTMS, using a Bruker APEX 4.7 T Fourier transform mass spectrometer, at a laser wavelength of 355 nm (neodymium-YAG laser), with 5 laser shots. Resolving power is 13,000. Sample deposition: 1 nmol of pentafluorobenzylated estradiol

in 1 μ l of ethyl acetate deposited onto a silver surface as a 2-mm spot size. Laser spot size is 100 μ m. (See Figure 8.)

5 Example 10. Analysis of compound 591

Compound 591 was synthesized by reacting 2,3,5,6-tetra-fluoro-4-(pentafluorophenyl)phenol sequentially with 3-bromo-2-butanone/ K_2CO_3 , piperazine/ K_2CO_3 and diphenylacetyl chloride. Detection of compound 591 by LI-EC(In)-TOF-MS is
10 shown in Figure 9. A 1 μ L solution containing 100 pmol of compound 591 in ethyl acetate was applied to an indium surface in a TOF-MS, giving a 2 mm spot size, and a nitrogen laser (100 μ m diameter spot size) was used.

Those with expertise in the field will recognize
15 further variations in the invention which are consistent with the disclosure.